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(57) Abstract

Insecticidal compositions are described comprising an essential oil obtained from a plant selected from the genera *Salvia*, *Artemisia*, *Citrus*, *Juniperus*, *Laurus*, *Myristica*, *Origanum*, *Piper* or *Aloysia*. The essential oil may be in a gel carrier, an aqueous alcoholic vehicle or a vehicle based on an alcohol/vegetable oil mixture. The compositions are particularly useful for ridding humans and animals of parasitic infestations such as lice and mites as well as for treatment of furnishings, clothing and plants.

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INSECTICIDAL COMPOSITIONS

The present invention relates to plant essential oils and extracts and their uses as insecticides both in medical and non-medical applications. In particular the invention relates to the oils and extracts of Spanish sage (*Salvia lavandulifolia*; syn. *S. lavandulaefolia*) and *S. officinalis* "petite feuille Banon" and other essential oils or plant extracts such as those from *Artemisia dracunculus* (Tarragon), *Citrus limon* (Lemon), *Juniperus communis* (Juniper), *Laurus nobilis* (Bay), *Myristica fragrans* (Nutmeg), *Origanum vulgare* (Oregano), *Piper cubeba* (Cubebs), *Aloysia gratissima* (Whitebrush) and species of *Salvia* other than *S. lavandulifolia* which have the ability to kill a range of ectoparasites in a range of formulations.

Sucking lice (*Anuplura*) are a common pest of humans and animals which have a very wide global distribution, and are often spread by physical contact. In humans, lice are of two main genera. Lice of the genus *Pediculus* include head lice (*Pediculus humanus capitis* syn. *P. capitis*) and clothing lice (*P. humanus humanus* syn. *P. corporis*), whilst the commonest lice of the genus *Pthirus* are the crab (or public) lice, *Pthirus pubis*.

Head lice are a very common problem in children, from whom the insects can then be spread to other family members. Infestations are by no means limited solely to children. In the strictest sense, lice are obligate parasites, and hence are unlikely to leave a host voluntarily, yet whether infestations can be spread by inanimate objects, such as combs or hairbrushes remains a controversial issue. Louse

- 2 -

infestations are usually accompanied by itching, although it is also possible that the insects could act as vectors for bacteria responsible for skin complaints, such as impetigo and scalp pyoderma (see Burgess (1995) *Advances in Parasitology* 36:271-342). In the UK, infestation rates were recorded as high as 23.1 % in primary school-aged children, and higher still (up to 30.3 %) in secondary schools. Other surveys suggest that one third of primary/junior school children are infested at least once per year (see Gratz (1997) *Human Lice- their prevalence, control and resistance to insecticides*, WHO).

There is evidence that a recrudescence of clothing lice, which are generally rarer than head lice in "developed" countries, is occurring (see Gratz (1997) *Human Lice- their prevalence, control and resistance to insecticides*, WHO). These insects have been linked to the transmission of trench fever, relapsing fever and typhus (see Van Der Lann and Smit (1996) *Nederlands Tijdschrift voor Geneeskunde* 140: 1912-1915).

Conventional treatment of head lice depends on application of insecticidal compositions and/or physical removal of lice and nits (lice eggs) by mechanical means (usually a fine-toothed comb). Insecticides used for treatment of head lice include organophosphates (e.g. malathion), pyrethroids, lindane and DDT. There are a number of problems associated with these compounds, including health concerns over exposure to organophosphates, development of resistance by lice (see Gratz (1997) *Human Lice- their prevalence, control and resistance to insecticides*, WHO) and an undesirable product formulation (e.g. unpleasant smell). Some

- 3 -

preparations also contain very high (>80% v/v) concentrations of alcohol, which has been linked to instances of allergenic reactions.

- 5 A number of plant-derived treatments for lice have been developed previously, ranging from *Stemosa tuberosa* and *Hyssop officinalis* extracts, quassia chips, pyrethrins, rotenone (from *Derris* or *Lonchocarpus* spp.) to a number of essential oils.
- 10 Essential oils which have been used, or suggested for use against lice, or which have been studied scientifically include *Pimpinella anisum* (Aniseed), *Cinnamomum zeylanicum* (Cinnamon), *Cymbopogon nardus* (Citronella), *Eucalyptus* spp. (Eucalyptus),
- 15 *Pelargonium graveolens* (Geranium), *Hyssop officinalis* (Hyssop), *Juniperus communis* (Juniper), *Lavandula officinalis* (Lavender), *Citrus limon* (Lemon), *Cypressus x leylandii* (Leyland cypress), *Myrtus communis* (Myrtle), *Myristica fragrans* (Nutmeg),
- 20 *Origanum vulgare* (Oregano), *Mentha x piperita* (Peppermint), *Pinus sylvestris* (Pine), *Thymus zygis* (Red thyme), *Rosemarinus officinalis* (Rosemary), *Melaleuca alternifolia* (Tea tree) and *Cananga odorata* (Ylang ylang). A number of these preparations have,
- 25 however, been found to be of limited efficacy or have been associated with mammalian toxicity (see Burgess (1995). *Advances in Parasitology* 36:271-342). US patents 5,227,163 and 5,411,992 to Eini claim that a number of essential oils, including sage, and also a
- 30 range of terpenoid compounds possess lice-repellent activity. Rosemary and Eucalyptus essential oils have similarly been demonstrated to possess repellent activity towards clothing lice (see Mumcuoglu et al (1996) *Entomologia Experimentalis et Applicata* 78:
- 35 309-314). Patent Application GB 2,341,091 also describes eucalyptus essential oil, in combination

- 4 -

with lavender and tea-tree essential oils, as a treatment for head lice. Combinations of essential oils, including rosemary, eucalyptus, tea-tree and lavender, as well as those from a number of other species, are also proposed as compositions suitable for lice treatment (W000/00213).

Ectoparasites of livestock, such as sheep, can detrimentally affect productivity of milk and meat, and the quality of wool and leather. Additionally, the welfare of animals infested with parasites can be seriously affected (see Bates (1999) in: Martin and Aitken (editors), *Diseases of Sheep* 3rd Ed., Chapter 45, Blackwell Science). Conventional treatment and control regimes are expensive to implement, and can be associated with health risks to the farmer.

Sheep scab results from infestation by *Psoroptes ovis* (sheep scab mite), and is usually treated by plunge dipping in washes containing organophosphates (such as diazinon or propetamphos) or synthetic pyrethroids (flumethrin or cypermethrin). Organophosphate dips have the advantage that a single immersion is sufficient for treatment (see Bates (1999) in: Martin and Aitken (editors), *Diseases of Sheep* 3rd Ed., Chapter 46, Blackwell Science), but have been linked to health concerns for farmers (e.g. see Rees (1996). *Occupational and Environmental Medicine* 53: 258-263), whereas pyrethroid dips need to be used twice at 14 day intervals for effective treatment. *P. ovis* is usually restricted to sheep, although *Psoroptic* mange in cattle has been reported, and is a major problem in mainland Europe and the United States of America. The ear mite, *P. cuniculi*, is a close relative of *P. ovis*, and is primarily associated with rabbit populations, although it has also been isolated from sheep, goats

- 5 -

and horses.

Chewing lice (*Bovicula ovis*) are another major ectoparasite of sheep, which can severely affect the quality of fleeces and hides. Treatment of lice is by similar means to the scab mite, by dipping in solutions containing organophosphates or synthetic pyrethroids. Pour-on treatments based on synthetic pyrethroids have been developed, but in Australia, resistance developed to these products within a few years of their release on to the market (see Bates (1999) in: Martin and Aitken (editors), *Diseases of Sheep* 3rd Ed., Chapter 45, Blackwell Science). The lipophilic nature of the components of the compositions described in the present invention make them ideal for development as pour-on treatments, for which lipophilicity is a key feature.

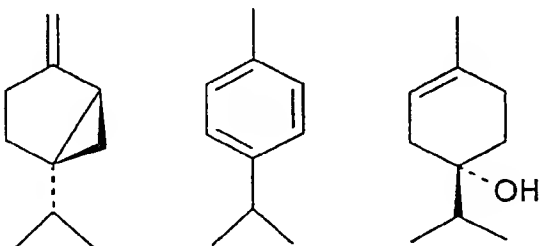
Sage contains a rich variety of chemicals, many of them terpenes and terpenoids. They include amorphene, aromadendrene, borneol, bornyl acetate, cadinene, camphene, camphor, β -caryophyllene, caryophyllene oxide, 1,8-cineole (eucalyptol), copaene, cubebene, *p*-cymene, geraniol, germacrene D, gurjunene, α -humulene, limonene, linalool, linalyl acetate, manool, myrcene, ocimene, palustrol, phellandrene, α -pinene, β -pinene, ~~sabinene, sabinyl acetate, spathulenol, terpenyl~~ acetate, terpinen-4-ol, terpinene, terpineol, terpinolene, α -thujone, β -thujone, viridifloral. Additional components of sage extracts could include (1R,5R)-epoxysalvial-4(14)-en-1-one, (2R,5E)-epoxycaryophyll-5-en-12-al, (2R,5E)-epoxycaryophyll-5-ene, (2S,5E)-epoxycaryophyll-5-en-12-al, 3-octanol, acetic-acid-ester, α -bisabolol, α -terpineol, α -terpinyl-acetate, arachidic acid, benzaldehyde, β -gurjuene, β -myrcene, β -sitosterol, butyric acid, caprylic acid, cerotinic acid, cis-3-hexen-1-ol, cis-

- 6 -

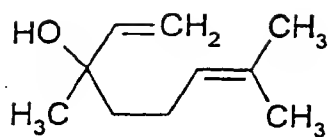
allo-ocimene, cis-beta-ocimene, cis-linalol-oxide, citral, citronellol, cuminaldehyde, delta-3-carene, furfurool, gamma-terpinene, geranial, geraniol, geranyl acetate, isospathulenol, lignoceric acid, linoleic acid, linolenic acid, n-nonanol, n-pentanol, neral, nerol, nerolidol, neryl acetate, oleanolic-acid, oleic-acid, palmitic acid, phellandrene, propionic acid-ester, rosmarinic acid, salvia-4(14)-en-1-one, sclareol, stearic acid, terpinen-4-ol, trans-allo-ocimene, trans- β -ocimene, trans- β -terpineol, trans-linalool oxide, ursolic acid, valeric acid-ester, 5-hydroxy-6,7,4'-trimethoxyflavone, 6,8-di-C-glucosylapigenin, 6-hydroxylutein-6,3'-dimethylether, 6-methoxy apigenin-7-glucoside, 6-methoxy apigenin-7-glucuronide, 6-methoxy luteolin-7-glucoside, 6-methoxy luteolin-7-glucuronide, apigenin-7-glucoside, apigenin-7-glucuronide, betulinic-acid, carnesol, chrysoeriol-7-glucuronide, luteolin-7-glucoside, luteolin-7-glucuronide, luteolin-7-glucuronide-3'-glucoside, luteolin diglucoside, picrosalvin, salvigenin, (E)-nerolidol, 2,6-dimethyl-10-(p-tolyl)-undeca-2,6-diene, 2-octanol, 3-octanol, allo-aromadendrene, α -amorphene, α -copaene, α -cubebene, α -gurjunene, α -humulene, α -muurolene, α -phellandrene, α -selinene, β -cyclocitral, cadin-1,4-diene, cis- α -bisabolene, δ -cadinene, γ -muurolene, isoamyl-acetate, isocaryophyllene, isopinocampone, methyl-perillate, myrtenol, myrtenyl acetate, perillaldehyde, perillyl acetate, perillyl alcohol, perillyl-butyrate, t-cadinol, trans- α -bergamotene, trans-calamenene and viridiflorene.

Sage essential oil samples which are most effective in killing parasites usually contain high concentrations of sabinene, p-cymene and/or terpinen-4-ol. Sabinene [(1R/S,5R/S)-1-isopropyl-4-methylenebicyclo[3.1.0]hexane] is a bicyclic monoterpene, of the formula $C_{10}H_{16}$, p-cymene [1-

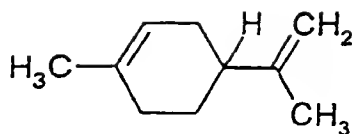
propyl-4-methylbenzene] is a monocyclic monoterpene hydrocarbon, of the formula $C_{10}H_{14}$, and terpinen-4-ol [(S)-1-isopropyl-4-methyl-3-cyclohexen-1-ol] is a cyclic monoterpene alcohol of the formula $C_{10}H_{18}O$, whose respective formulae can be represented thus:



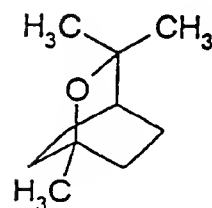
Other compounds tested in the present research include linalool [3,7-dimethyl-1,6-octadien-3-ol], limonene [(R/S)-4-isopropenyl-1-methylcyclohexane], 1,8-cineole [eucalyptol; 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane], camphor [1,7,7-trimethylbicyclo[2.2.1]heptan-2-one], α -terpineol [(R/S)-p-menth-1-en-8-ol], α -pinene [(1R/S, 5 R/S)-2,6,6-trimethyl-bicyclo[3.1.1]hept-2-ene], β -pinene [(1R/S, 5 R/S)-6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane], borneol [endo-(1R/S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol], myrcene [7-methyl-3-methylen-1,6-octadiene], camphene [(1R/S)-2,2-dimethyl-3-methylenebicyclo[2.2.1]heptane] and β -caryophyllene [trans-(1R,9S)-8-methylene-4,11,11-trimethylbicyclo[7.2.0]undec-4-ene]. Their formulae can be represented thus:



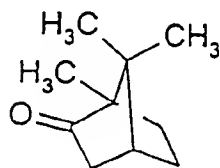
Linalool



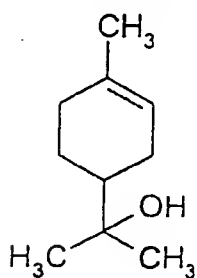
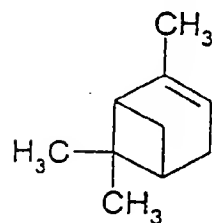
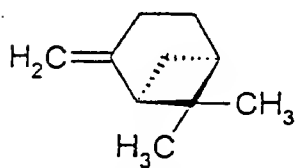
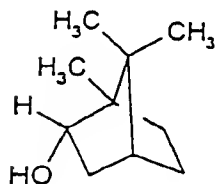
limonene



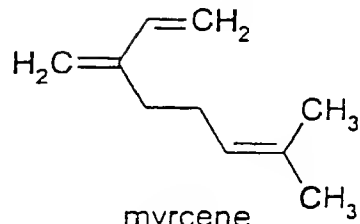
1,8-cineole



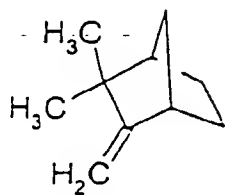
camphor

 α -terpineol α -pinene β -pinene

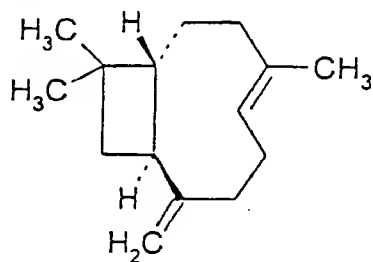
borneol



myrcene



camphene

 β -caryophyllene

- 9 -

The parasite-killing activity of these compounds described in the present invention is previously unreported, although they have has been shown to possess other biological activities. For example, US
5 patent 5,635,184 to Camano describes potent antibacterial activity of the essential oil of *Schinus molle*, which contains significant amounts of sabinene. A composition based on volatile monoterpene compounds, one of which could include sabinene, has been
10 described as possessing activity against house- and leopard mites (see Arakawa Chem Ind Ltd (1992) Japanese Patent 0413914A).

It is also desirable for these oils to have low
15 concentrations of thujone and sabinyol acetate, which have been linked to mammalian toxicity (see Tisserand (1995) *Essential Oil Safety- A Guide for Health Care Professionals*; Fournier et al. (1993) *Plant Medica* 59: 96-97). Spanish sage (*Salvia lavandulifolia*) is
20 particularly desirable in this regard, due to its low thujone content, and is classified as safe to use, being unlikely to cause toxicity, irritation or sensitisation. Despite the presence of thujone in other Sage essential oils, these have been determined
25 to be of lower toxicity than expected on the basis of their thujone content alone, and are unlikely to cause irritation or sensitisation (see Tisserand (1990) *Essential Oil Safety Data Manual*). The same source also indicates that, for dermal application, essential
30 oils of similar potential toxicity as thujone-rich sage oil could be applied twice weekly at concentrations between 1-2 and 3-5 % by volume. These values are all above the final concentration of sage oil in an aqueous-alcoholic carrier, as described in
35 the present invention.

- 10 -

It is therefore an object of the present invention to provide treatments for parasitic infestations of humans and animals, which are pleasant to use, effective, of low mammalian toxicity, and are unlikely to lead to the development of side-effects or adverse reactions. It is a further object of the invention to provide compositions for treating insect parasitic infestations of fabrics including clothing and furnishings. It is yet a further object of the invention to provide compositions for treatment of parasitic infestations of plants.

In accordance with a first aspect the invention provides a composition comprising an essential oil in a gel carrier, said essential oil being obtained from a plant selected from the genera *Salvia*, *Artemisia*, *Citrus*, *Juniperus*, *Laurus*, *Myristica*, *Origanum*, *Piper* or *Aloysia*, said composition being for use in the treatment of a human or animal having a parasitic insect infestation.

The gel may be based on agar, agarose, gelatin, or a synthetic gelling agent and may be subject to dilution with water and/or alcohol, preferably, isopropyl alcohol. As used herein the term "synthetic gelling agent" refers to a material having the properties of a gel but which is not found in nature.

In accordance with a second aspect the invention provides a composition comprising an essential oil in an aqueous alcoholic vehicle in which the vehicle comprises from about 0.1% up to about 20% alcohol v/v with water and wherein said essential oil is obtained from a plant selected from *Salvia*, *Artemisia*, *Citrus*, *Juniperus*, *Laurus*, *Myristica*, *Origanum*, *Piper* or *Aloysia*, said composition being for use in the

- 11 -

treatment of a human or animal having a parasitic insect infestation.

5 In accordance with a third aspect the invention provides a composition comprising an essential oil in a vehicle comprising an alcohol/vegetable oil mixture wherein said alcohol is present in said vehicle in an amount of about 0.1% up to about 20% v/v and wherein
10 said essential oil is obtained from a plant selected from the genera *Salvia*, *Artemisia*, *Citrus*, *Juniperus*, *Laurus*, *Myristica*, *Origanum*, *Piper* or *Aloysia*, said composition being for use in the treatment of an animal having a parasitic insect infestation.

15 In accordance with a fourth aspect the invention provides a composition comprising an essential oil in a gel carrier, said essential oil being obtained from one be more of the following: *Pelargonium*, *Cymbopagon*, *Pimpinella*, *Myrtus* (Cretian, Morrocan, orange)
20 *Lavandula*, *Pinus*, *Melaleucas*, *Cinnamomum*, *Apium*, *Thymus*, *Hyssopus*, *Rosmarinus*, *Cananga*, *Mentha*, *Eucalyptus* or *Vitex*.

The properties of the gel carrier may be as already
25 described above.

In accordance with a fifth aspect the invention provides a composition comprising the alkaloid galanthamine for use in the treatment of a human or
30 animal having a parasitic insect infestation. Preferably, the galathamine is comprised in an extract from the plant genus *Narcissus*.

The above described compositions are useful for the
35 killing and/or repelling of parasitic insects in a variety of different contexts. They may be used for

- 12 -

treatment of humans having a parasitic insect infestation, for example head lice or pubic lice. The gel formulations of the first and fourth aspects of the invention are particularly useful for this purpose and may be formulated for topical use such as for hair gels or mousse, for example.

Non-gel formulations such as those described in the second and third aspects of the invention are also suitable for the treatment of humans, for example as a shampoo, but are also well-suited to the treatment of domestic and agricultural animals, as well as insect infested clothing, furnishings and plants. For such applications they may be formulated into sprays, dips or pour-on solutions. Formulations using vegetable oil/alcohol vehicles such as set forth in the third aspect of the invention are particularly suited to the topical treatment of animals suffering from insect infestations.

Further preferred embodiments of the compositions and methods of the invention are disclosed herein below and in the accompanying examples and claims.

As aforesaid various individual components of essential oils of the plant genera described above which are terpenes or terpinoid compounds have been shown to possess insecticidal activity, particularly against lice.

Thus, in accordance with yet a further aspect the invention provides compositions comprising any one of/or combination of, the terpenes and terpenoids shown to be insecticidally active according to Figures 7 to 10 of this application for any one of the above described insecticidal uses, whether or not said terpene or terpinoid is comprised in a plant essential

- 13 -

oil or not. Preferred compositions are those comprising sabinene, p-cymene, β -pinene, mycrene, limonene and terpinen-4-ol or compositions comprising combinations of two or more thereof. Particularly preferred are compositions comprising sabinine in combination with limonene and/or terpinen-4-ol.

Also preferred are compositions having concentrations of an insecticially active terpene or terpinoid which is higher than would be found in the essential oil of one of the aforementioned plant genera. For example, the level of terpenes and terpinoids in essential oils may be enhanced by mixing the essential oil with ethanol and partitioning the resulting solution with known volumes of water. Preferred elevated terpene and terpenoid compositions comprise from about 4 to about 25% of the terpene or terpinoid or mixtures thereof. A particularly preferred composition comprises from about 4 to about 25% sabinene.

Compositions comprising terpenes and terpinoids as described above may be formulated in any of the ways described herein and are suitable for any of the various applications described herein.

The present invention is based on the discovery that essential oils can effectively kill insects, especially parasitic insects such as ectoparasites. Certain oils, including those from *Salvia* species and other plant species containing relatively high concentrations of sabinene p-cymene, β -pinene, myrcene or terpinen-4-ol have been found, surprisingly, to be most effective. and therefore of considerable commercial potential. Species of *Salvia* suitable for use in the present invention include *Salvia aethiopis*, *Salvia amissa*, *Salvia apiana*, *Salvia argentea*, *Salvia arizonica*, *Salvia azurea*, *Salvia ballotiflora*, *Salvia*

- 14 -

blodgettii, *Salvia brandegei*, *Salvia carduacea*, *Salvia*
carnosa, *Salvia chapmanii*, *Salvia chia*, *Salvia*
clevelandii, *Salvia coccinea*, *Salvia columbariae*,
Salvia davidsonii, *Salvia divinorum*, *Salvia*
5 *dolichantha*, *Salvia dorrii*, *Salvia earlei*, *Salvia*
engelmannii, *Salvia eremostachya*, *Salvia farinacea*,
Salvia funerea, *Salvia glutinosa*, *Salvia grahamii*,
Salvia greatae, *Salvia greggii*, *Salvia henryi*, *Salvia*
hispanica, *Salvia lancifolia*, *Salvia lemmonii*, *Salvia*
10 *leptophylla*, *Salvia leucophylla*, *Salvia longistyla*,
Salvia lycioides, *Salvia lyrata*, *Salvia mellifera*,
Salvia micrantha, *Salvia microphylla*, *Salvia misella*,
Salvia mohavensis, *Salvia munzii*, *Salvia nemorosa*,
Salvia nutans, *Salvia occidentalis*, *Salvia*
15 *officinalis*, *Salvia pachyphylla*, *Salvia parryi*, *Salvia*
penstemonoides, *Salvia pinguifolia*, *Salvia pitcheri*,
Salvia potus, *Salvia pratensis*, *Salvia privoides*,
Salvia ramosissima, *Salvia reflexa*, *Salvia regla*,
Salvia riparia, *Salvia roemeriana*, *Salvia sclarea*,
20 *Salvia serotina*, *Salvia sonomensis*, *Salvia spathacea*,
Salvia splendens, *Salvia subincisa*, *Salvia summa*,
Salvia texana, *Salvia thomasiana*, *Salvia tiliifolia*,
Salvia urticifolia, *Salvia vaseyi*, *Salvia verbenacea*,
Salvia verticillata, *Salvia vinacea*, *Salvia virgata*,
25 *Salvia X bernardina*, *Salvia X palmeri*, *Salvia X*
superba and *Salvia X sylvestris*. Particularly
preferred are *Salvia lavandulifolia* or *S. officinalis*.
Other species suitable for use in the present
invention include *Artemesia dracumculus* (Tarragon),
30 *Citrus*, in particular *Citrus limon* (lemon), *Juniperus*
communis (Juniper), *Laurus nobilis* (Bay), *Myristica*
fragrans (Nutmeg), *Origanum Vulgare* (Oregano), *Piper*
cubeba (Cubebbs) and *Aloysia gratissima* (Whitebrush).
The oils can be formulated into both aqueous and non-
35 aqueous formulations which remain active against lice.
The preferred formulation, in which the oil maintains

- 15 -

significant activity at relatively low concentrations is based on a gel, in which the essential oil is dispersed. This has the advantage over mixed aqueous alcoholic formulations to which sensitive individuals may react adversely due to their alcohol content. In a mixed alcoholic-aqueous carrier, concentrations of oil required to kill human lice are comparable to those of the (organophosphate-based) active components in commercial head lice treatments.

The present invention therefore may be summarised as an effective dose of an essential oil or plant extract, preferably Spanish sage essential oil in the concentration range 0.1-50 % (w/v), in a suitable carrier vehicle based on a carrier oil, such as vegetable oil, an aqueous-alcoholic vehicle, such as IPA-water with IPA in the concentration range 0-50 %, or, preferably in a gel formulation, such as Lubrajel TW (available from United Guardian Inc of 230, Marcus Blvd, Hauppauge, New York, 11788), containing water (0-66 % v/v), IPA (0-20 %) and/or additional plant extracts, as required. For example, the preferred formulation for treatment of human lice is Lubrajel TW diluted 1:2 (v/v) with water, to which 4 % (w/v) Spanish sage essential oil and 0.5 % (w/v) decolourised Aloe vera gel are added.

The ability of sabinene-containing essential oils, and in particular those from plants of the genus *Salvia* (sage), to kill insects, and in particular parasitic lice and mites is demonstrated in the examples herein. Essential oils, and in particular the oils from *lavandulifolia* and *S. officinalis* "petite feuille Banon", typically containing high concentrations of sabinene, and low concentrations of thujone, immobilise both head- and clothing lice almost immediately on contact, and remain highly active when

- 16 -

appropriately formulated. As described above the present invention incorporates a number of embodiments comprising a range of formulation types including carrier oils, mixed water-alcohol vehicles and gel forms suitable for topical application to the head or skin of humans, application to furnishings and clothing and to plants. The same preparations are also effective treatments for animal ectoparasites, for example sheep parasites, including *Psoroptes* (mite) and *Bovicula* (louse) species.

It is further demonstrated herein that a number of terpene and terpenoid components of the sage oil synergistically interact, and kill ectoparasites at lower doses than when applied individually in an *in vitro* model. (see Example 4).

The terpene hydrocarbon content of essential oil samples can be elevated by mixing with ethanol, and partitioning the resulting solution with known volumes of water. The most effective ratio of solvents for these experiments is 5 volumes of ethanol:water (3:2) per volume of essential oil, although a range of ratios, from 1:1:1 to 6:4:1 (ethanol:water:essential oil) are also effective. When the partitioning is repeated, the resulting oil shows an increased content of terpene hydrocarbons.

Previously, the lice-repellency activity of *Salvia sclarea* essential oil and terpene or terpenoid components characteristic of this, and related oils, has been described (see Eini et al. 1995 US Patent 5,411,992). The lice- and mite-killing activity of sage essential oils, and their chemical components is previously unreported.

- 17 -

Activity of essential oils and terpene or terpenoid components of essential oils was tested using an *in vitro* model system. Test solutions were prepared by accurately dissolving and mixing essential oils or terpenoid components in a range of carrier solutions, including water, isopropanol (propan-2-ol; IPA), water-IPA mixtures in the range 10-90 % by volume IPA and inactive vegetable oils, such as grape seed oil and a range of gel formulations, containing 0-66% water and/or 0-20% IPA. Clothing lice (*Pediculus humanus humanus*), head lice (*Pediculus humanus capitis*) or sheep biting lice (*Bovicula ovis*) were then transferred to small volumes (typically 1-2 ml) of these test solutions, in glass containers, and were left in contact with the solutions for 10 minutes. During this time the solutions were occasionally shaken gently to ensure adequate contact between the lice and the test solution. After 10 minutes, the lice were removed, and placed on filter paper to remove excess treatment.

Exposure of lice to test substances in this way (treatments or controls) resulted in the lice being temporarily immobilised. The lice were then monitored for signs of physical activity for a period of up to 90 minutes. Lice exposed to non-active test substances or controls typically regained activity within 5 minutes of their removal from the test solution; those which failed to regain any activity within a post-treatment period of 30-90 minutes were classified as dead.

Due to their smaller size, parasitic mites (*Psoroptes ovis* or *P. cuniculi*) were treated by adding droplets of test solution to the parasites. The time taken for the mites to stop moving in the test solution was

- 18 -

taken as an indication of the toxicity of the test substance. In all other respects, the experiments were performed similarly to those used to study parasitic lice.

5

Each experiment was performed at a range of concentrations of the test substance, to determine the dose-response of these materials. Activity of the treatments was expressed as an LD₅₀, which was the concentration of essential oil or terpenoid compound sufficient to kill half of the parasites exposed to that treatment.

In the following non-limiting Examples reference is made to the following figures.

15

Figure 1 shows the effect of concentration of sage oil in vegetable oil carrier on clothing lice recovery (%);

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Figure 2 shows the effect of concentration of sage oil in 20% (v/v) IPA-water on clothing lice recovery (%);

25

Figure 3 shows data obtained on testing activity on clothing lice of essential oils from a number of plant genera at 20 mg/ml (2% w/v) in an aqueous gel;

30

Figure 4 shows data obtained on testing activity on clothing lice of essential oils from a number of plant genera at 40 mg/l (4% w/v) in an aqueous gel;

35

Figure 5 shows the effect of sage oil in a vegetable oil carrier against ear mites (*Psoroptes cuniculi*) over a range of sage oil concentrations;

Figure 6 shows the effect of sage oil in a carrier of IPA (20% v/v) in water against ear mites over a range

- 19 -

of sage oil concentrations;

Figure 7 shows effect of a range of terpene and terpenoid components of essential oils in a vegetable oil carrier at 125 - 200 mg/ml against human parasitic lice;

Figure 8 shows the effect of a range of terpene and terpenoid components of essential oils at 25 mg/ml in Lubrajel TW (1 part) and water (2 parts) against human parasitic lice;

Figure 9 shows the effect of a range of terpene and terpenoid components of essential oils at 10 mg/ml in Lubrajel TW (1 part) and water (2 parts) against human parasitic lice; and

Figure 10 shows the effect of a range of terpene and terpenoid components of essential oils in a carrier vegetable oil against *Psoroptes cuniculi*.

Example 1a

Activity of sabinene-containing essential oils against human parasitic lice

At high concentrations, sage essential oil led to gross morphological disruption of the human lice, characterised by abdominal swelling, and the development of an intense red coloration in the body and limbs. When dissolved in an inert (no activity against lice) carrier oil, the LD₅₀ for sage essential oil containing as little as 5 % sabinene was in the concentration range of 250-300 mgml⁻¹ (Table 1, FIG. 1). In a carrier consisting of IPA (20 % v/v) in water, however, the LD₅₀ of the same oil was much lower, at between 3-4 mgml⁻¹ (Table 2, FIG. 2). In gel-based formulations, LD₅₀ values were below 10 mgml⁻¹

- 20 -

¹, in the case of gel diluted 1:2 (v/v) with water (Table 3) or with undiluted gel supplemented with 20 % (w/v) IPA. The diluted gel was selected as the preferred formulation, due to the low LD₅₀ of sage essential oil it contains, and the absence of alcohol from the formulation, to which certain individuals can be sensitive. Specifically, the preferred formulation is based on Lubrajel TW (a synthetic gel based on glyceryl polymethacrylate and propylene glycol), diluted with deionized water 1:2 (v/v), to which the essential oil, and other components are added, as required.

As head lice (*Pediculus humanus capitis*) were slightly more sensitive to the sage oil formulations than clothing lice (*P. humanus humanus*) (Tables 2 and 4), subsequent experiments were performed using clothing lice as the model system, as concentrations of oils effective against these lice would also be effective against head lice.

Table 1: Effect of concentration of sage oil in vegetable oil carrier on clothing lice recovery (%)

| Concentration (mgml ⁻¹) | Time post-treatment (minutes) | | | | | |
|--|-------------------------------|----|----|----|-----|-----|
| | 0 | 2 | 5 | 10 | 15 | 30 |
| 0 | 0 | 60 | 80 | 80 | 100 | 100 |
| 50 | 0 | 40 | 60 | 60 | 60 | 80 |
| 100 | 0 | 40 | 60 | 60 | 60 | 80 |
| 150 | 0 | 60 | 60 | 60 | 60 | 60 |
| 200 | 0 | 40 | 60 | 60 | 60 | 60 |
| 250 | 0 | 20 | 40 | 40 | 60 | 60 |
| 300 | 0 | 0 | 0 | 0 | 20 | 20 |

Table 2: Effect of concentration of sage oil in 20% (v/v) IPA-water on clothing lice recovery (%)

| Concentration (mg ml ⁻¹) | Time post-treatment (minutes) | | | | | |
|---|-------------------------------|----|-----|-----|-----|-----|
| | 0 | 2 | 5 | 10 | 15 | 30 |
| 0 | 0 | 0 | 100 | 100 | 100 | 100 |
| 1 | 0 | 0 | 100 | 100 | 100 | 100 |
| 2 | 0 | 33 | 100 | 100 | 100 | 100 |
| 3 | 0 | 33 | 33 | 33 | 33 | 100 |
| 4 | 0 | 0 | 33 | 33 | 33 | 33 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 3: Effect of concentration of sage oil in gel diluted 1:2 with water on head lice recovery (%)

| Concentration (mgml ⁻¹) | Time post-treatment (min) | | | | |
|--|---------------------------|----|-----|-----|-----|
| | 0 | 2 | 5 | 10 | 30 |
| 0 | 0 | 60 | 100 | 100 | 100 |
| 10 | 0 | 0 | 0 | 0 | 20 |
| 50 | 0 | 0 | 0 | 0 | 0 |
| 100 | 0 | 0 | 0 | 0 | 0 |

Table 4: Effect of concentration of sage oil in 20% (v/v) IPA-water on head lice recovery (%)

| Concentration (mgml ⁻¹) | Time post-treatment (min) | | | | |
|--|---------------------------|----|----|-----|-----|
| | 0 | 2 | 5 | 10 | 30 |
| Control | 0 | 25 | 50 | 100 | 100 |
| 1 | 0 | 33 | 67 | 67 | 33 |
| 2 | 0 | 29 | 43 | 57 | 29 |
| 3 | 0 | 25 | 50 | 50 | 50 |
| 4 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0 |

Addition of isopropanol (IPA) to vegetable carrier oil, in the range 0-20% (v/v) led to increased activity of the sage oil components, with the IPA itself having no direct effect of the lice over this concentration range. At concentrations of 20% IPA (v/v) in inert (grapeseed) oil, 66-100% of lice treated in this manner recovered activity within 10 minutes. With the addition of a sub-LD₅₀ dose (150 mgml⁻¹) of sage oil to the grapeseed oil, however, a clear dose response was apparent with increasing concentrations of IPA (Table 5).

Table 5: Effect of concentration of IPA in vegetable oil containing 150 mgml⁻¹ sage essential oil on clothing lice recovery (%)

| IPA concentration (% v/v) | Time post-treatment (minutes) | | | | |
|------------------------------|-------------------------------|----|----|----|----|
| | 0 | 5 | 10 | 15 | 30 |
| 5 | 0 | 57 | 71 | 71 | 71 |
| 10 | 0 | 33 | 50 | 50 | 50 |
| 15 | 0 | 0 | 0 | 0 | 13 |
| 20 | 0 | 0 | 0 | 0 | 0 |

In the case of the gel-based formulations, addition of IPA had varying effects, depending on the gel strength. In strong gels (undiluted with water), activity increased with the addition of IPA over the range 0-20 % (v/v), but in weaker gels, (diluted 1:1 or 1:2 with water), addition of IPA over the same range reduced activity (Table 6).

Table 6: Effect of gel strength and IPA content of formulations containing 10 mgml⁻¹ sage essential oil on clothing lice recovery after 30 minutes (%)

| Gel strength | Lice recovery (%) | |
|-------------------|-------------------|--------------|
| | No IPA | 20 IPA (v/v) |
| Undiluted | 100 | 20 |
| Diluted 1:1 (v/v) | 60 | 60 |
| Diluted 1:2 (v/v) | 20 | 100 |

A number of essential oil samples were studied for activity, and data from gas chromatographic analysis

of the most active oil samples (*S. officinalis* "petite feuille Banon" and *Salvia lavandulifolia*), is given in tables 7 and 8.

Table 7: Identified chemical components of the essential oil of *Salvia officinalis* "petite feuille Banon"

| Name | Percentage peak area |
|--|----------------------|
| a-pinene | 4.26 |
| camphene | 5.61 |
| sabinene/ β -pinene | 8.03 |
| myrcene | 3.16 |
| limonene/1,8-cineole/ <i>p</i> -cymene | 33.80 |
| linalool | 0.32 |
| α -thujone | 0.47 |
| β -thujone | 0.22 |
| camphor | 9.51 |
| borneol/isoborneol | 2.29 |
| terpinen-4-ol | 1.71 |
| α -terpineol | n.d. |
| linalyl acetate | n.d. |
| β -caryophyllene | 3.90 |
| n.d. | Not determined |

Table 8: Identified chemical components of the essential oil of *Salvia lavandulifolia*

| Name | Percentage peak area |
|--|----------------------|
| a-pinene | 12.69 |
| camphene | 9.39 |
| sabinene/ β -pinene | 2.34 |
| myrcene | 0.97 |
| limonene/1,8-cineole/ <i>p</i> -cymene | 37.46 |
| linalool | 0.78 |
| α -thujone | 0.17 |
| β -thujone | n.d. |
| camphor | 22.98 |
| borneol/isoborneol | 1.62 |
| terpinen-4-ol | 0.15 |
| α -terpineol | 1.08 |
| linalyl acetate | 1.35 |
| β -caryophyllene | 0.94 |
| n.d. | Not determined |

When incorporated into the preferred formulation, Spanish sage essential oil was more effective as a pediculicide than any of the other essential oils tested, including those known from the prior art.

- 5 After exposure to sage essential oil at a concentration of 20 mgml⁻¹ (2 % w/v), clothing lice started a (very limited) recovery after 50 minutes, later than all the other oil samples tested (FIG. 3).

At 40 mgml⁻¹ (4 % w/v), lice showed no recovery when exposed to sage oil, or a number of other oils (FIG. 4), but, due to the greater activity at 2% (w/v), sage oil was clearly the most active oil tested.

5

A number of oils in addition to sage oils were also tested in the aqueous-alcoholic and vegetable oil carrier systems. At a concentrations of between 2 and 10 mgml⁻¹ in the IPA-water carrier, the essential oils from cubebs (*Piper cubeba*) and lemon (*Citrus limon*) were most active, killing 100% and 66% of clothing lice, as assessed 60 minutes after treatment. In the vegetable oil carrier system (at concentrations of 300 mgml⁻¹), nutmeg (*Myristica fragrans*), lemon (*Citrus* 15 *limon*) and tarragon (*Artemisia dracunculus*) were also all active against at least 66% of the clothing lice treated. Concentrations of *Salvia lavandulifolia* essential oil of as little as 4 mgml⁻¹ in 20% IPA-water were sufficient to kill all treated lice in some 20 experiments, compared to 6 mgml⁻¹ for the essential oil of tea tree (*Melaleuca alternifolia*), which is commonly used as an "alternative" treatment for head lice, indicating sage oil is more potent than tea-tree oil against these lice.

25

Example 1b

Activity of sabinene-containing essential oils in alcohol-water and carrier oils against parasites of non-human species

30

When dissolved in an inert carrier oil, the LD₅₀ for sage essential oil containing as little as 5 % sabinene against ear mites (*Psoroptes cuniculi*) was in the concentration range of 50-100 mgml⁻¹ (FIG. 5). In

- 25 -

a carrier consisting of IPA (20 % v/v) in water, however, the LD₅₀ of the same oil was lower, at between 4-5 mgml⁻¹ (FIG. 6). Against biting lice (*Bovicula ovis*), sage oil in an inert oil carrier at 300 mgml⁻¹ or in 20% IPA-water at 5 mgml⁻¹ was highly effective at killing lice after an exposure time of 10 minutes.

Example 2a

Activity of terpene and terpenoid essential oil components in carrier oil against human parasitic lice.

A number of terpene and terpenoid components of the essential oil of sage are commercially available. A range of these compounds were tested for relative activity by dissolving/mixing the compounds at equimolar concentrations in inert vegetable carrier oil. For the comparative studies, all components were prepared to a concentration of 0.97M (approximately 125-200 mgml⁻¹, depending on molecular weight). To avoid only partial solubility in predominantly aqueous carrier media, vegetable oil was selected as the carrier was these studies; all compounds were fully soluble in this medium.

These experiments demonstrated that there was great variation in the activity of the essential oil components against clothing lice, ranging from complete activity (sabinene) through to no detectable activity (1,8-cineole). The other components showed intermediate activity, with limonene and caryophyllene the most active components excluding sabinene (FIG. 7, Table 9). The same compounds were also prepared in a

gel formulation containing Lubrajel TW (1 part) and water (2 parts) at 25 mgml⁻¹ (FIG. 8), and those displaying complete activity at this concentration were then tested again at 10 mgml⁻¹ (FIG. 9).

5

Table 9: Effect of sage oil components on recovery (%) of clothing lice
(all compounds at equimolar concentrations in carrier oil)

| Compound | Time post-treatment (min) | | | | | |
|-----------------|---------------------------|----|-----|-----|-----|-----|
| | 0 | 2 | 5 | 10 | 15 | 20 |
| borneol | 0 | 0 | 50 | 50 | 50 | 50 |
| camphene | 0 | 50 | 50 | 50 | 50 | 50 |
| camphor | 0 | 0 | 50 | 50 | 50 | 50 |
| b-caryophyllene | 0 | 0 | 0 | 0 | 25 | 50 |
| 1,8-cineole | 0 | 50 | 100 | 100 | 100 | 100 |
| p-cymene | 0 | 0 | 50 | 50 | 50 | 50 |
| limonene | 0 | 0 | 0 | 25 | 25 | 25 |
| linalool | 0 | 0 | 50 | 100 | 100 | 100 |
| myrcene | 0 | 0 | 0 | 50 | 50 | 50 |
| α-pinene | 0 | 50 | 50 | 50 | 50 | 50 |
| sabinene | 0 | 0 | 0 | 0 | 0 | 0 |
| terpinen-4-ol | 0 | 0 | 0 | 50 | 50 | 50 |
| terpineol | 0 | 50 | 50 | 50 | 50 | 50 |
| Carrier oil | 0 | 0 | 100 | 100 | 100 | 100 |

Example 2b

Activity of terpene and terpenoid essential oil components in carrier oil against parasites of non-human species.

5

Against *Psoroptes cuniculi*, as with the human parasitic lice, there was great variation in the activity of the essential oil components, ranging from complete activity (linalool) through to no detectable activity (caryophyllene). The other components showed intermediate activity, with both limonene and sabinene displaying complete activity within 5 minutes of application to the mites (FIG. 10).

15

Example 3

Activity of sage oil compared to conventional treatments.

- 27 -

Initial studies indicate that the use of sage oil at 5 mgml⁻¹ in 20% IPA-water is more effective in killing lice than synthetic insecticides used in commercial treatments on a weight-weight basis in the same solvent system. For example, clothing lice treated with 5 mgml⁻¹ sage oil in 20% IPA-water showed no recovery of activity, whereas lice treated similarly with 5 mgml⁻¹ malathion or permethrin in 20% IPA-water showed signs of recovery within 1 hour. The legs and antennae of approximately 80% of malathion-treated lice showed twitching motion 40 minutes after treatment, whilst lice exposed to permethrin were able to move (albeit slowly) within 45 minutes of treatment. In both these cases, all the treated lice were able to undertake locomotion within 48 hours of treatment. No such activity was observed in lice treated with 5 mgml⁻¹ sage oil.

Example 4

Interaction between isolated monoterpene essential oil components in carrier oil

To test whether isolated terpene and terpenoid components of sage essential oils were able to interact with each other, and kill lice at concentrations below their LD₅₀ values when tested in isolation, sub-lethal concentrations of the compounds listed in tables 7 and 8 were prepared in carrier oil. In practice, the concentrations tested were as follows: myrcene (0.24 M), *b*-caryophyllene (0.24 M), *p*-cymene (0.25 M), terpinen-4-ol (0.32 M), linalool (0.32 M), sabinene (0.37 M), *α*-terpineol (0.43 M), 1,8-cineole (0.43 M), limonene (0.48 M) and *α*-pinene (0.73 M). At these concentrations, clothing lice

- 28 -

rapidly recovered activity, typically within 5 minutes of treatment.

Combinations of sabinene with limonene, sabinene with α -terpineol and limonene with terpinen-4-ol and all
5 demonstrated increased activity against lice, compared to the isolated compounds. In these combinations of compounds, the lice failed to recover activity within 5 minutes, whereas the lice exposed to the isolated
10 compounds at sub-lethal concentrations were fully active by this time.

Against ear mites (*Psoroptes cuniculi*), combinations of sabinene with α -terpineol, linalool with α -terpineol and linalool with *p*-cymene were all more
15 active than their monoterpene components applied to the mites in isolation.

Example 5

Other natural compounds active against lice.

20 In addition to sabinene-containing essential oils, extracts from the bulbs of plants of the genus *Narcissus* were also determined to display potent activity against lice. Galanthamine, an alkaloid found in such extracts, was determined to be
25 particularly active in this regard, and was completely effective against lice at concentrations of 5 mgml⁻¹ in 20 % IPA (v/v) in water. The lice-killing activity of galanthamine was possibly due to its potent ability to inhibit acetylcholinesterase, which was determined
30 using spectrophotometric methods (M. Ryan).

Example 6

Efficacy of a formulated product against parasites *in vivo*.

- 29 -

A formulated product containing 4% (w/v) Spanish sage essential oil and 0.5% Aloe vera gel (10x concentrated) in Lubrajel TW (1 part)-water (2 parts) was applied to the hair of a volunteer infested with head lice. After initial assessment of the infestation, 60 ml of the treatment was applied to the scalp, and covered with a shower cap for a period of 20 minutes. The cap was then removed, and the product removed by careful washing. The volunteer's hair was combed with a fine-toothed comb to remove dead lice. The procedure was then repeated after 7 days, to allow any eggs not killed by the initial treatment to be hatch. Following the second application, and after a period of 7 further days, there was no sign of lice infestation in the volunteer.

Example 7

Efficacy of a formulated product against agricultural pests

A formulated product containing 0.5-2.0% (w/v) Spanish sage essential oil in 20% (v/v) IPA-water was applied to aphids, both *in situ* (on the plant on which they were feeding), and under *in vitro* conditions. When sprayed onto the aphids, the formulated product quickly immobilised the aphids, and, 60 minutes after application, they remained inactive. Application of the formulated product, in the form of an aerosol, or as a solution, was 100% effective in killing aphids under these conditions.

30

CLAIMS:

1. A composition comprising an essential oil in a gel carrier, said essential oil being obtained from a plant selected from the genera *Salvia*, *Artemisia*, *Citrus*, *Juniperus*, *Laurus*, *Myristica*, *Origanum*, *Piper* or *Aloysia*, said composition being for use in the treatment of a human or animal having a parasitic insect infestation.

2. A composition as claimed in claim 1 wherein the concentration of essential oil is from about 0.1 to about 50% w/v of the composition.

3. A composition as claimed in claim 2 wherein the concentration of essential oil is from about 0.1 to about 8% w/v of the composition.

4. A composition as claimed in claim 3 wherein the concentration of essential oil is about 4% w/v of the composition.

5. A composition as claimed in any preceding claim wherein the gel is based on agar, agarose, gelatin or a synthetic gelling agent.

6. A composition as claimed in claim 5 wherein the synthetic gelling agent is a gel based on glyceryl polymethacrylate and propylene glycol.

7. A composition as claimed in claim 4 or claim 5 wherein the gel is a carbomer.

8. A composition as claimed in any one of claims

- 31 -

5 to 7 wherein the gelling agent contains about 0.1 to about 95%, preferably about 0.1 to about 66% water v/v and/or alcohol, preferably isopropyl alcohol (IPA) at about 0.1% to about 20% v/v.

5

9. A composition as claimed in any preceding claim wherein said essential oil is obtained from *Salvia lavandulifolia* or *Salvia officinalis*.

10

10. A composition as claimed in any one of claims 1 to 8 wherein said essential oil is obtained from a plant of the genera *Citrus*.

15

11. A composition as claimed in any one of claims 1 to 8 which includes essential oil obtained from the plant genera *Salvia* and *Citrus*.

20

12. A composition as claimed in claim 9 which comprises the essential oil of *Salvia lavandulifolia* at about 4% w/v dispersed in a gel diluted about 1:2 v/v with water.

25

13. A composition as claimed in any preceding claim comprising an anti-pruritic agent.

14. A composition as claimed in claim 13 wherein said anti-pruritic agent is *Aloe vera*.

30

15. A composition as claimed in claim 14 wherein said composition includes *Aloe vera* gel.

16. A composition as claimed in claim 15 which comprises about 0.1 to about 5.0% w/v *Aloe vera* gel.

- 32 -

17. A composition as claimed in any one of claims 12 to 16 which comprises about 0.5% w/v *Aloe vera* gel.

5 18. Use of a gel and essential oil selected from the essential oils defined in claim 1 in the manufacture of a composition for the treatment of a human or animal having a parasitic insect infestation.

10 19. A method of treating a human or animal suffering from a parasitic insect infestation comprising applying to said human or animal a composition as claimed in any one of claims 1 to 17.

15 20. A composition comprising an essential oil in an aqueous alcoholic vehicle wherein said vehicle comprises from about 0.1% up to about 20% alcohol v/v with water and wherein said essential oil is obtained from a plant selected from the genera *Salvia*,
20 *Artemisia*, *Citrus*, *Juniperus*, *Laurus*, *Myristica*, *Origanum*, *Piper* or *Aloysia*, said composition being for use in the treatment of a human or animal having a parasitic insect infestation.

25 21. A composition as claimed in claim 20 wherein said vehicle comprises about 20% v/v alcohol with water.

30 22. A composition comprising an essential oil in a vehicle comprising an alcohol/vegetable oil mixture wherein said alcohol is present in said vehicle in an amount of about 0.1% up to about 20% v/v and wherein said essential oil is obtained from a plant selected from the genera *Salvia*, *Artemisia*, *Citrus*, *Juniperus*,

- 33 -

Laurus, Myristica, Origanum, Piper or *Aloysia* said composition being for use in the treatment of an animal having a parasitic insect infestation.

5 23. A composition as claimed in claim 22 wherein said vehicle comprises about 20% alcohol v/v.

10 24. A composition as claimed in any one of claims 20 to 23 wherein the alcohol is isopropyl alcohol.

15 25. A composition as claimed in any one of claims 20 to 24 wherein said essential oil is obtained from *Salvia lavandulifolia* or *Salvia officinalis*.

 26. A composition as claimed in any one of claims 20 to 24 wherein said essential oil is obtained from a plant of the genera *Citrus*.

20 27. A composition as claimed in any one of claims 20 to 24 wherein said essential oil is obtained from the genera *Salvia* and *Citrus*.

25 28. A composition as claimed in any one of claims 20 to 27 which is formulated as a dip, spray or pour-on treatment.

30 29. Use of an aqueous alcoholic vehicle and an essential oil as defined in claim 20 in the manufacture of a composition for the treatment of a human or animal having a parasitic insect infestation.

 30. A method of treating a human or animal suffering from a parasitic insect infestation

- 34 -

comprising applying to said human or animal a composition as claimed in any one of claims 20, 21, 24, 25, 26, 27 and 28.

5 31. Use of a vegetable oil/alcohol vehicle and an essential oil as claimed in claim 22 in the manufacture of a medicament for the treatment of an animal having a parasitic insect infestation.

10 32. A method of treating an animal having a parasitic insect infestation comprising applying to said animal a composition as claimed in any of claims 22 to 28.

15 33. A composition for use as claimed in any one of claims 1 to 17 and 20 to 28 wherein said parasitic insects are selected from lice, lice eggs, mites, fleas or parasites associated with blowfly strike.

20 34. A composition as claimed in claim 33 wherein said parasitic insects are selected from head lice (*Pediculus humanus capitis*, syn. *P. capitis*), clothing lice (*Pediculus humanus humanus* syn. *P. corporis*), pubic lice (*Phthirus pubis*), biting lice (*Bovicula ovis*), scab mite (*Psoroptes ovis*), ear mite (*Psoroptes cuniculi*), dust mites (primarily of the genus *Dermatophagoides*, pig mites, cat fleas (*Ctenocephalides felis*), dog fleas (*C. canis*), horse fleas and *Lucilia* or *Chrysomya* species.

30

35. A method suitable for treating furnishing or clothing to kill parasitic insects which comprises exposing said furnishings to a composition of the type defined in any one of claims 20, 21, 24, 25, 26, 27

- 35 -

and 29.

36. A method suitable for treating plants to kill parasitic insects which comprises applying to
5 said plants a composition of the type defined in any one of claims 20, 21, 24, 25, 26, 27 and 29.

37. A method as claimed in claim 36 wherein said parasitic insects are selected from the genera *Aphis*,
10 *Chilo*, *Dysderus*, *Megoura*, *Musca*, *Pieris*, *Nilaparvata*, *Nephotettix*, *Tetranychus*, *Trialeurodes*, *Thysanoptera* or *Lepidoptera*.

38. A composition comprising an essential oil in
15 a gel carrier, said essential oil being obtained from a plant selected from the genera *Petergonium*, *Cymbopogan*, *Pimpinella*, *Myrtus* (cretian, Morrocan, organe), *Lavandula*, *Pinus*, *Melaleuca*, *Cinnamomum*, *Apium*, *Thymus*, *Hyssopus*, *Rosmellus*, *Cananga*, *Mentle*,
20 *Eucalyptus* or *Vitex*.

39. A composition as claimed in claim 38 wherein said gel is as defined in any one of claims 5 to 7.

25 40. A composition comprising the alkaloid galanthamine for use in the treatment of a human or animal having a parasitic insect infestation.

30 41. A composition for use as claimed in claim 40 wherein said composition comprises an extract of a plant of the genus *Narcissus*.

42. A composition for use as claimed in claim 40 or 41 wherein said galanthamine or extract is

dissolved in an oil-based medium, a water/alcohol based medium or is formulated as a hair conditioner or shampoo or as a gel, dip or pour on treatment.

5 43. A composition as claimed in claim 42 wherein said gel is as defined in any one of claims 5 to 7.

10 44. A composition as claimed in claim 42 wherein said galanthamine or extract is carried in a vehicle comprising from about 0.1 to about 20% alcohol (v/v).

15 45. A composition as claimed in claim 44 wherein said vehicle comprises about 20% v/v isopropyl alcohol.

20 46. Use of galanthamine or an extract of *Narcissus* in the manufacture of a composition for treating a human or animal suffering from a parasitic insect infestation.

25 47. A method of treating a human or animal suffering from a parasitic insect infestation which comprises applying to said human or animal a composition as claimed in any one of claims 40 to 45.

30 48. A composition for use as claimed in any one of claims 40 to 45 wherein said parasitic insects are selected from lice, lice eggs, mites, fleas or parasites associated with blowfly strike.

 49. A composition for use as claimed in claim 48 wherein said parasitic insects are selected from head lice (*Pediculus humanus capitis*, syn. *P. capitis*), clothing lice (*Pediculus humanus humanus* syn. *P.*

- 37 -

corporis), pubic lice (*Pthirus pubis*), biting lice (*Bovicula ovis*), scab mite (*Psoroptes ovis*), ear mite (*Psoroptes cuniculi*), dust mites (primarily of the genus *Dermatophagoides*), pig mites, cat fleas (5 *Ctenocephalalides felis*), dog fleas (*C. canis*), horse fleas and *Lucilia* or *Chrysomya* species.

50. A method suitable for treating furnishing or clothing to kill parasitic insects which comprises
10 exposing said furnishings or clothing to a composition of the type defined in any one of claims 40 to 45, 48 and 49.

51. A method suitable for treating plants to
15 kill parasitic insects which comprises applying to said plants a composition of the type defined in any one of claims 40 to 45, 48 and 49.

52. A method as claimed in claim 36 to 42
20 wherein said parasitic insects are selected from the genera *Aphis*, *Chilo*, *Dysdercus*, *Megoura*, *Musca*, *Pieris*, *Nilaparvata*, *Nephotettix*, *Tetranychus*, *Trialeurodes*, *Thysanoptera* and *Lepidoptera*.

25 53. A composition comprising a gel carrier and one of, or a combination of two or more of, the terpenes and terpenoids shown to have insecticidal activity according to the data of Figures 7 to 10 for use in treating humans, animals, clothing and
30 furnishings and plants having a parasitic insect infestation.

54. A composition as claimed in claim 53 wherein said terpene or terpenoid is selected from one or more

- 38 -

of sabinene, p-cymere, β -pinene, mycrene, limonine and terpinen-4-ol.

55. A composition as claimed in claim 53 which
5 comprises a combination of sabinene with limonene and/or terpinen-4-ol.

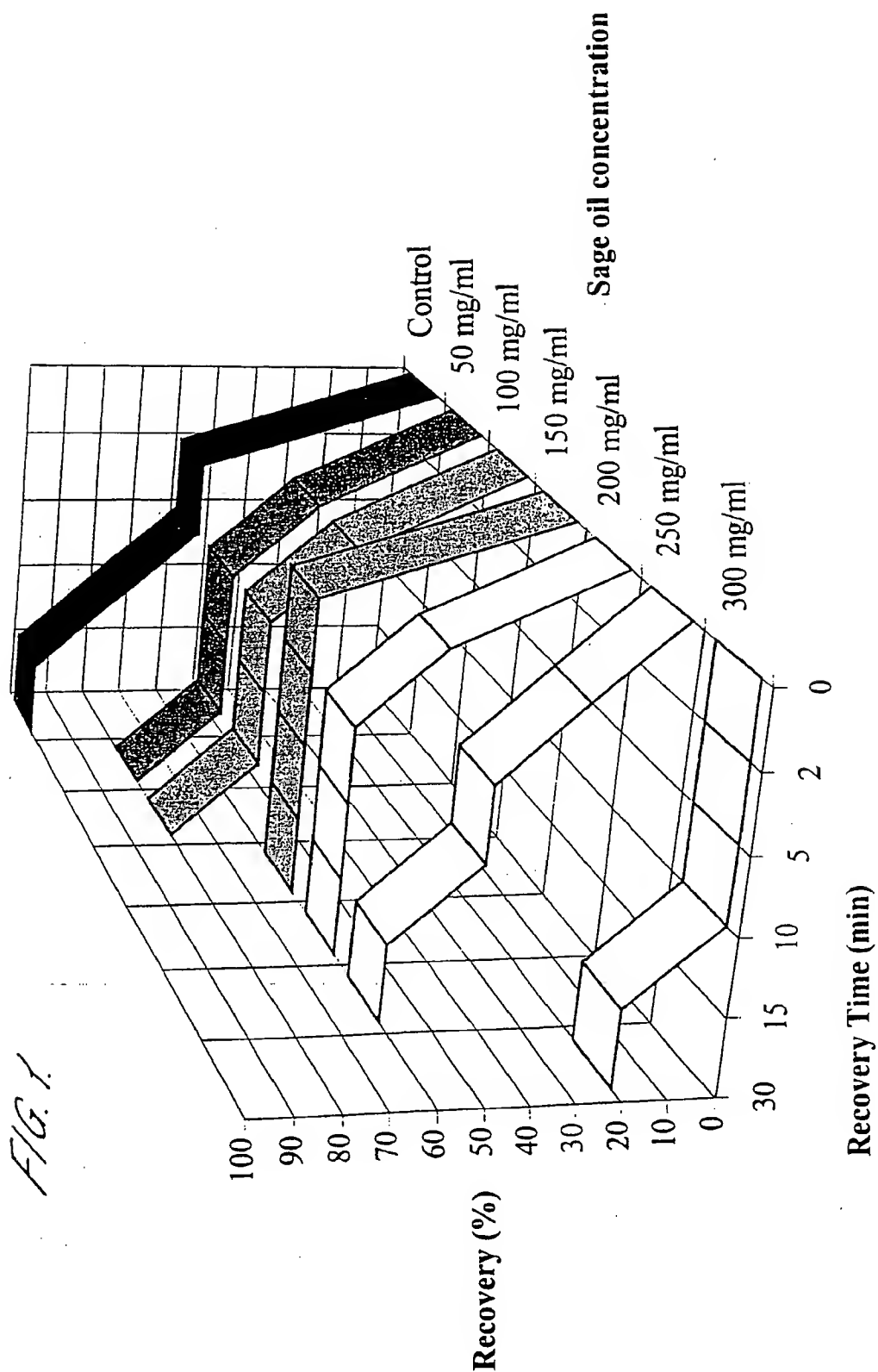
56. A composition comprising one of the terpenes and terpenoids shown to have insecticidal activity
10 according to the data shown in Figures 7 to 10 at a concentration of about 4% by weight or above for use in treating humans, animals, clothing and furnishings and plants having a parasitic insect infestation.

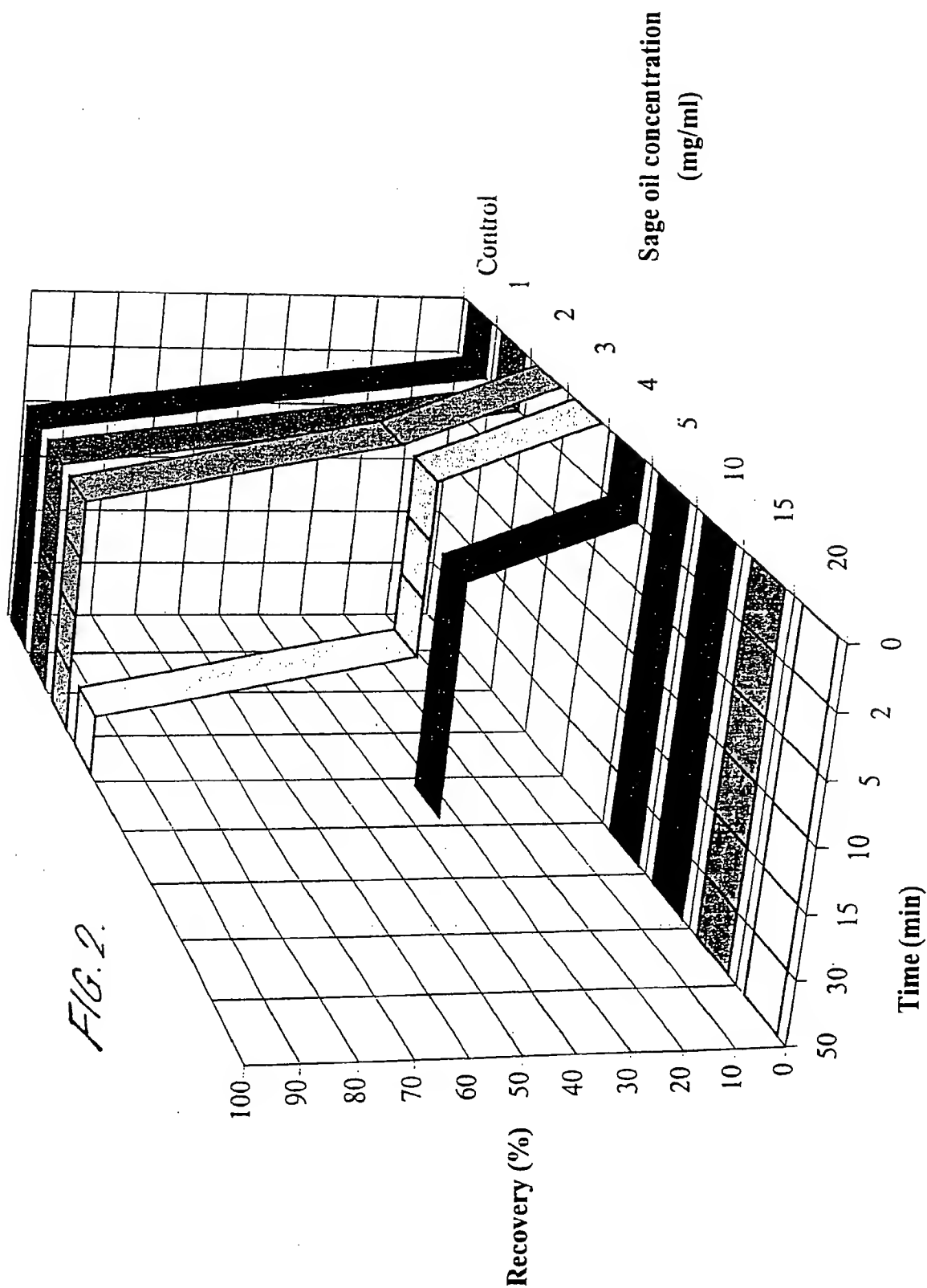
15 57. The composition of claim 56 modified to include one or more further terpene or terpenoid compounds from the list shown in Figures 7 to 10.

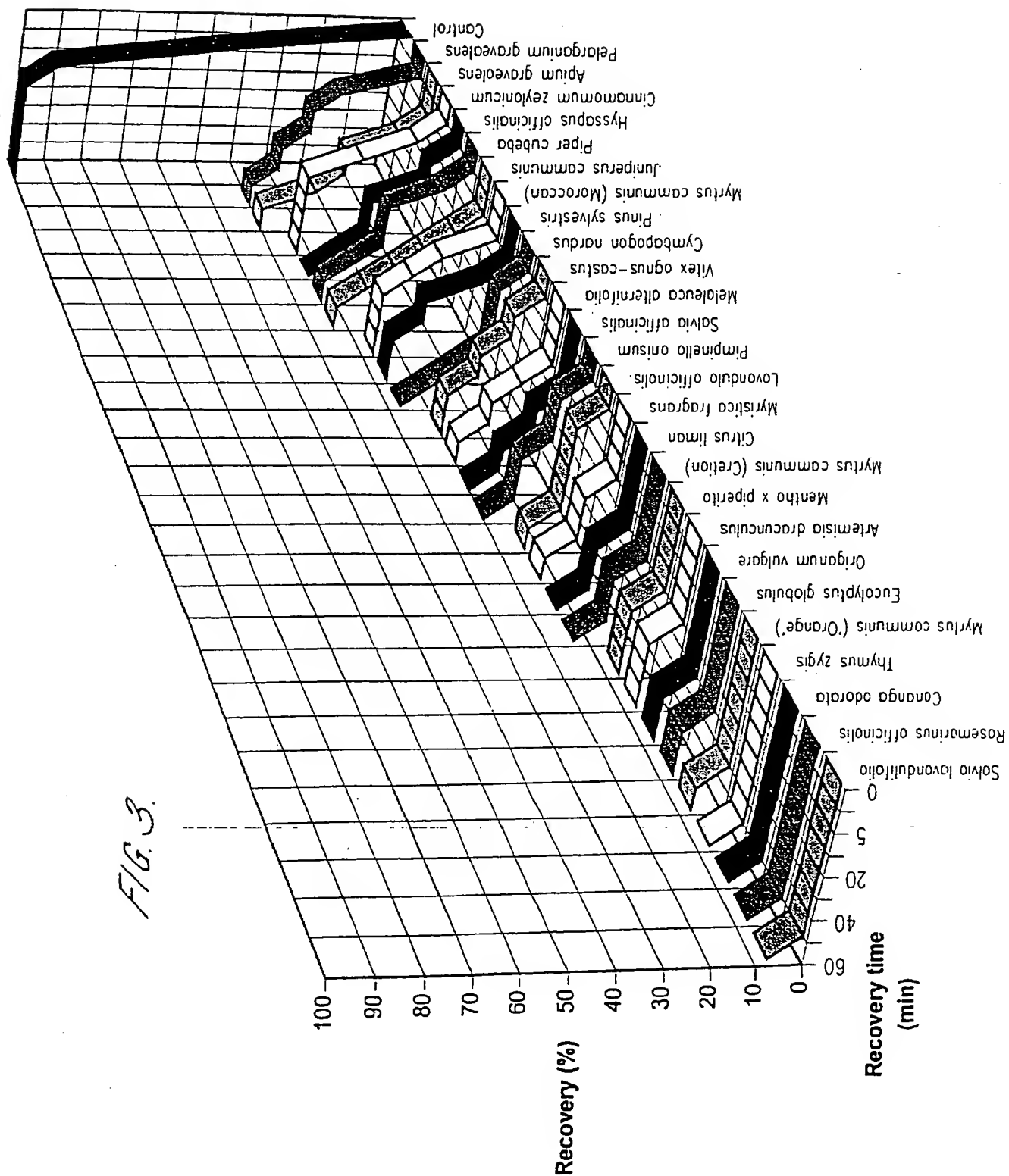
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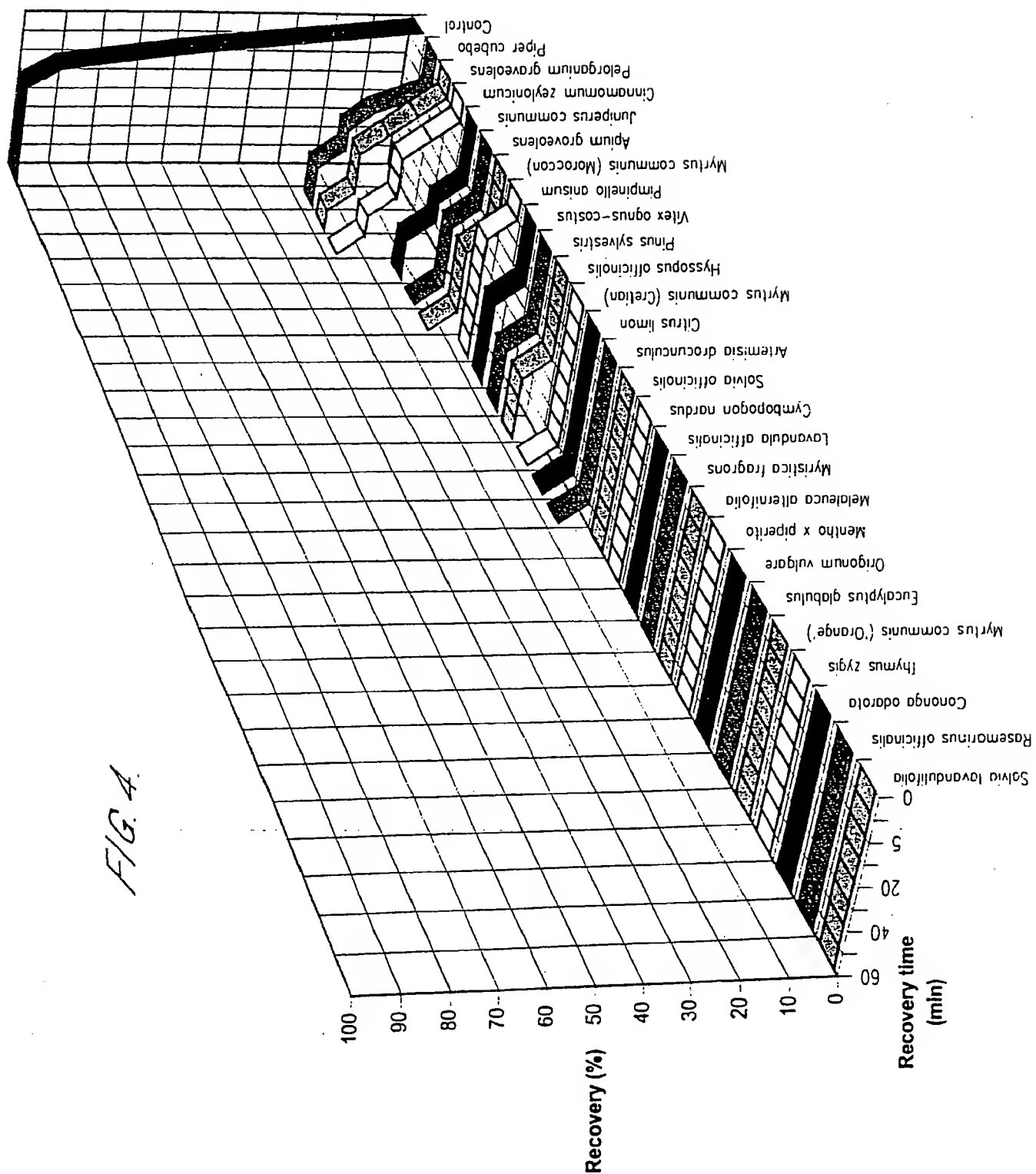
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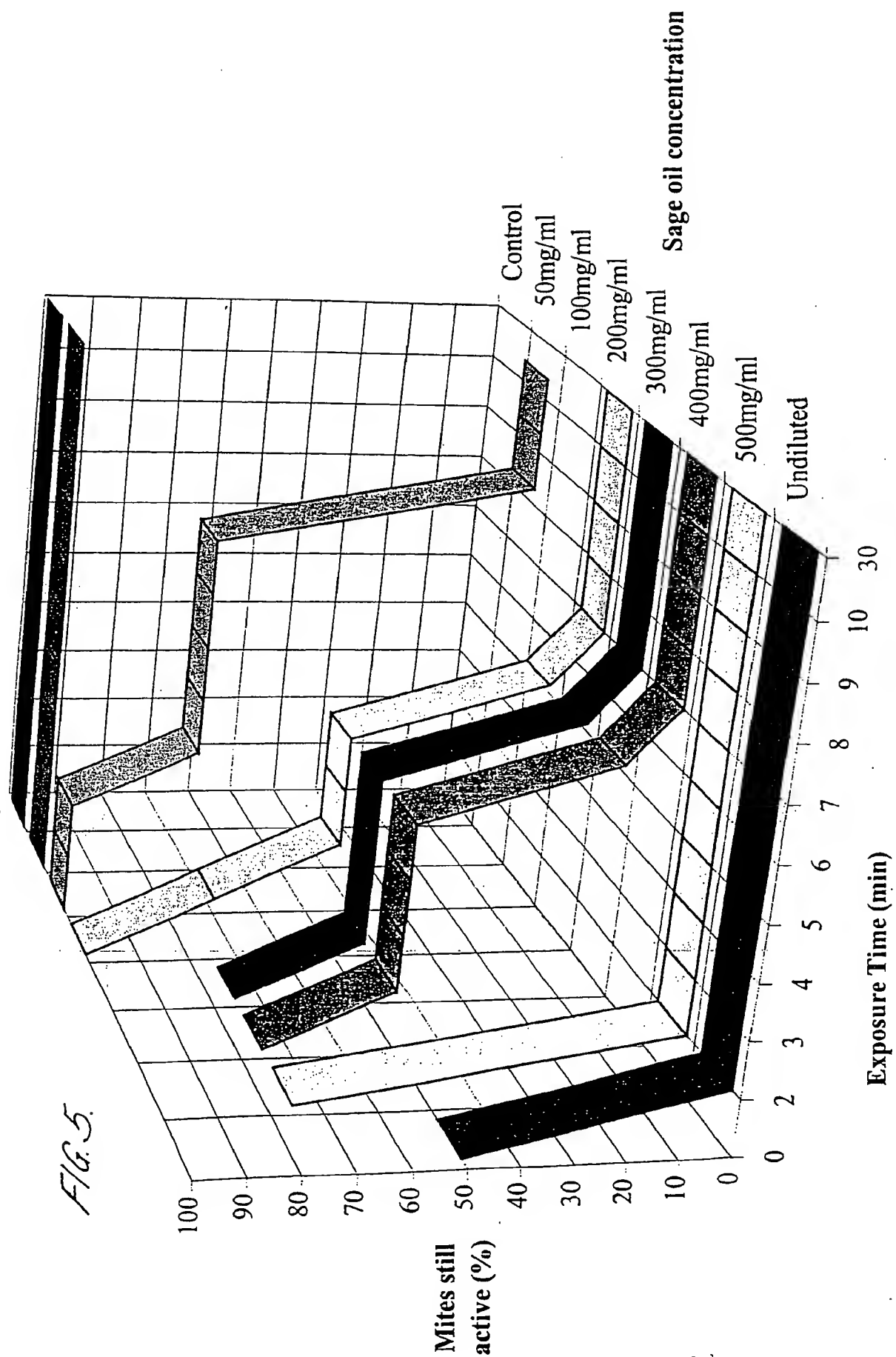
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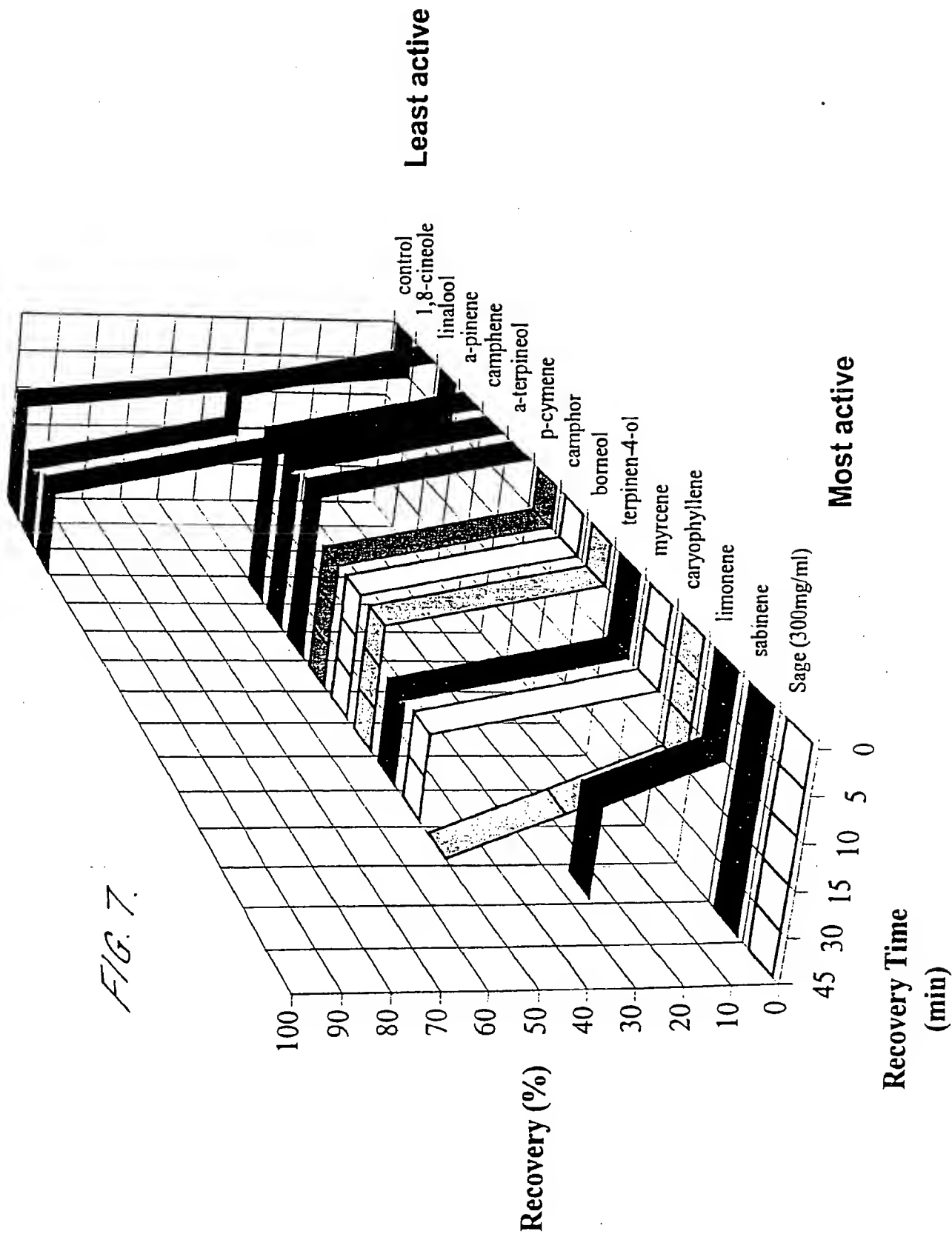
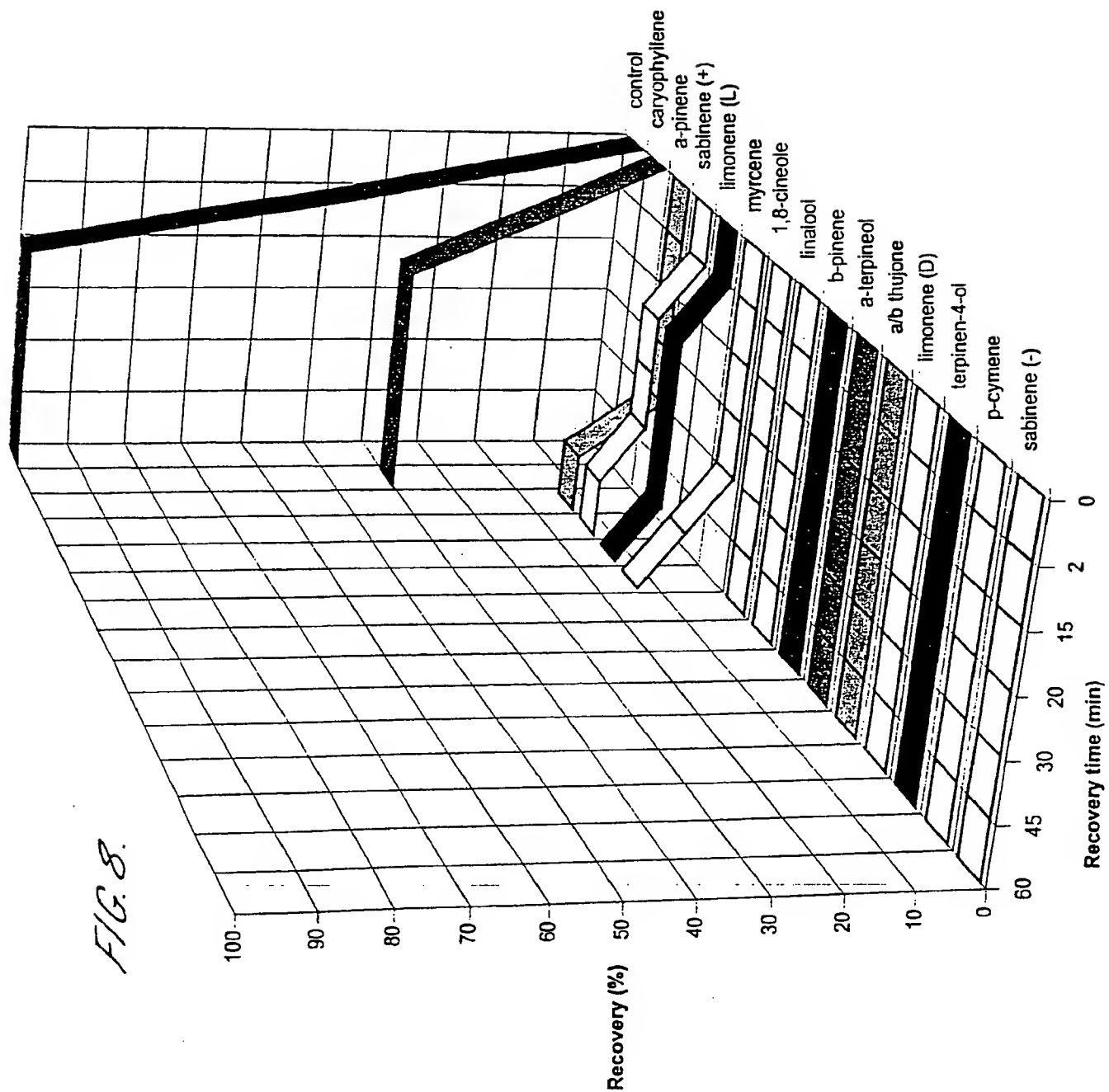
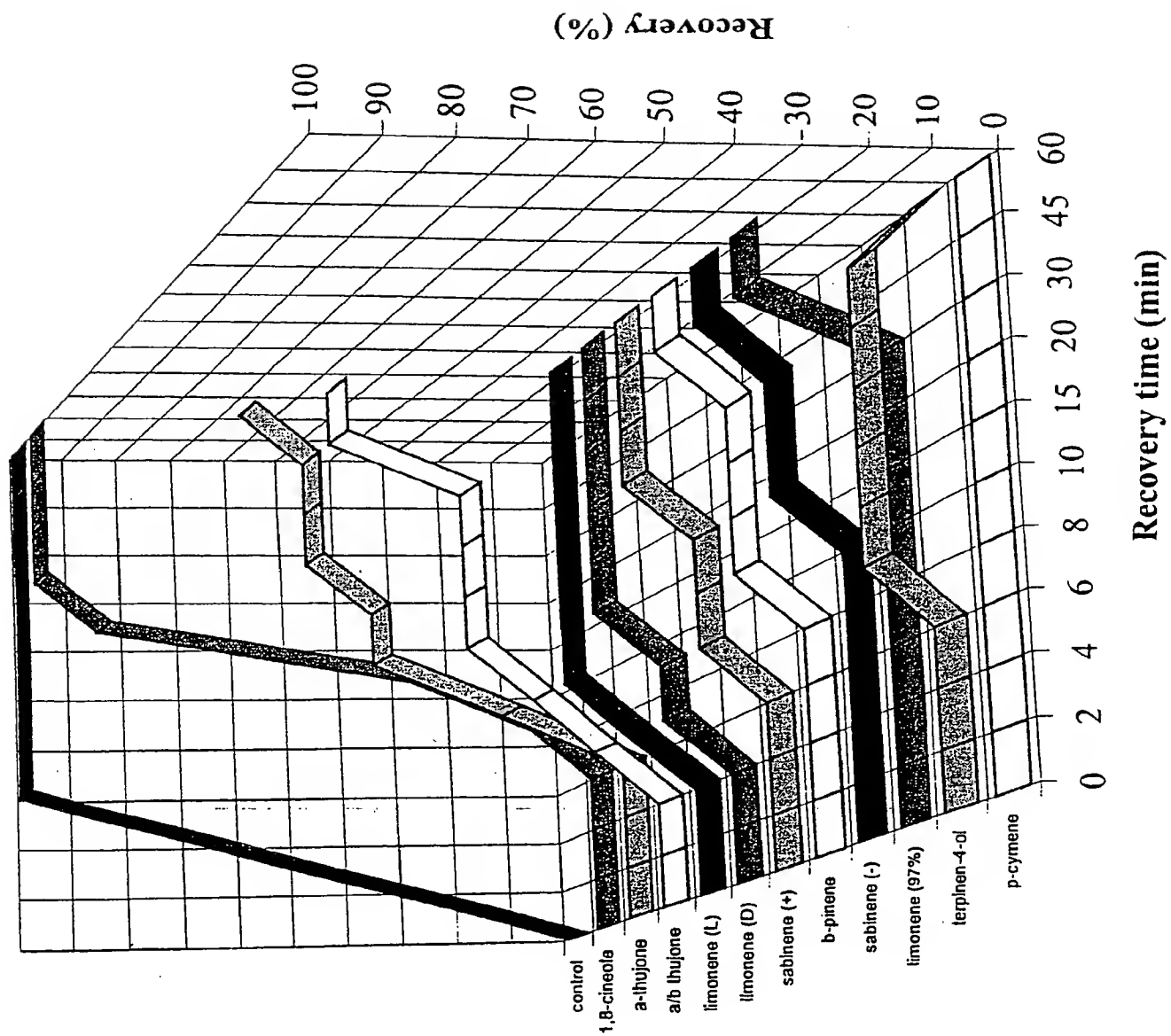
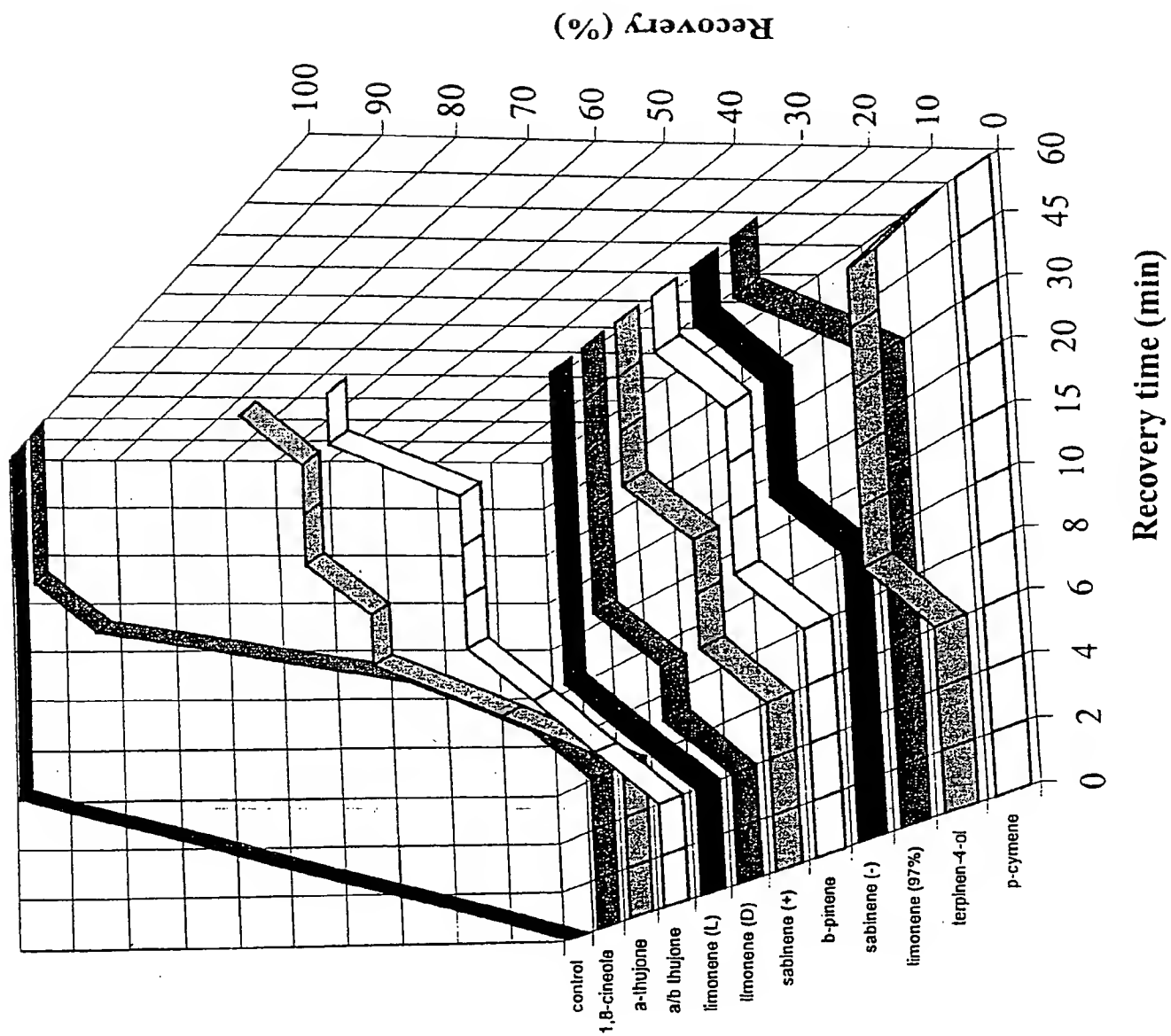
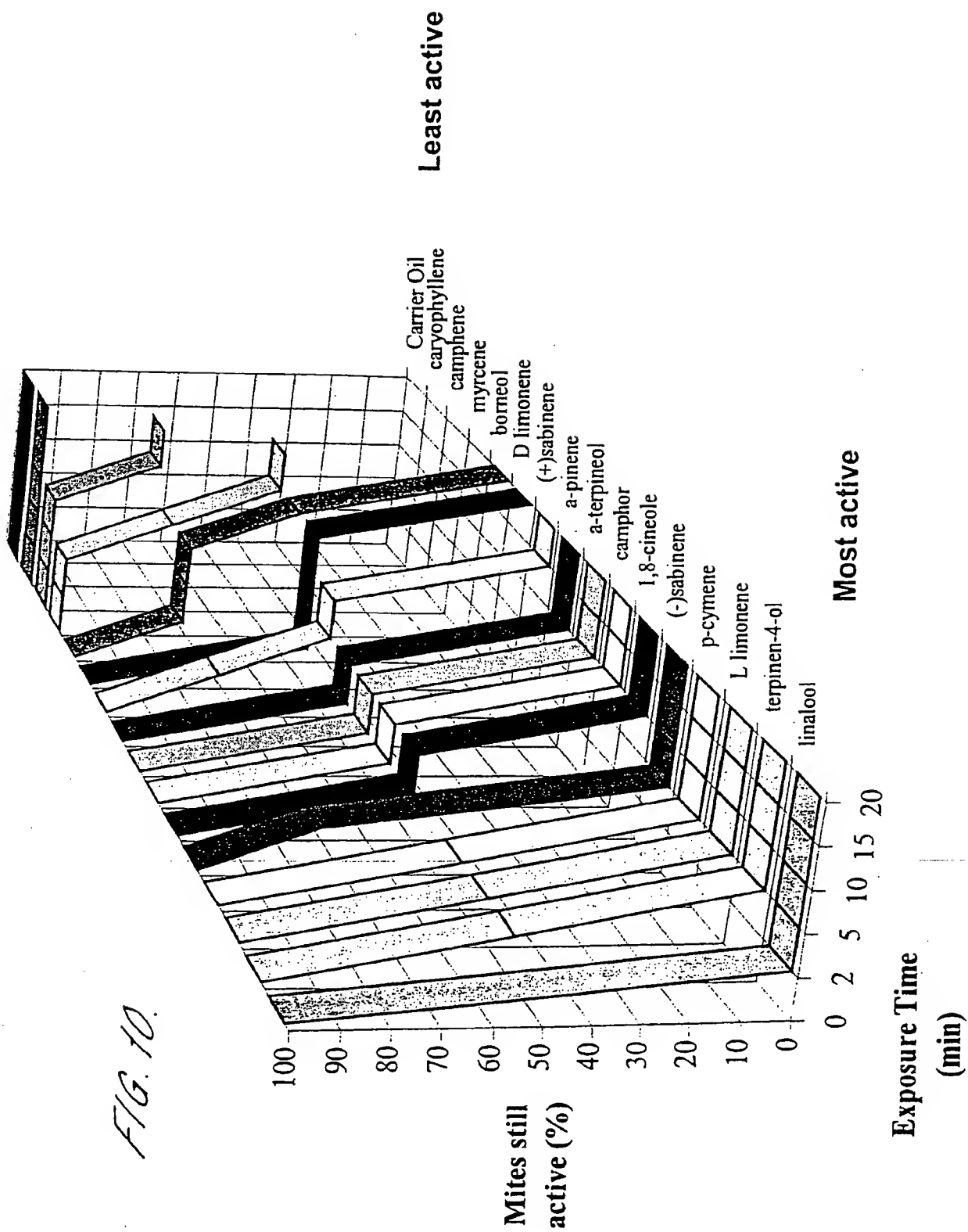


FIG. 8.





10 / 10



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(54) Title: INSECTICIDAL COMPOSITIONS

(57) Abstract: Insecticidal compositions are described comprising an essential oil obtained from a plant selected from the genera *Salvia*, *Artemisia*, *Citrus*, *Juniperus*, *Laurus*, *Myristica*, *Origanum*, *Piper* or *Aloysia*. The essential oil may be in a gel carrier, an aqueous alcoholic vehicle or a vehicle based on an alcohol/vegetable oil mixture. The compositions are particularly useful for ridding humans and animals of parasitic infestations such as lice and mites as well as for treatment of furnishings, clothing and plants.

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INTERNATIONAL SEARCH REPORT

Inter. .onal Application No

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A01N65/00 A01N25/04 A01N25/02 A61K35/78 A61K7/40

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A01N A61K.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, EPO-Internal, PAJ

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| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| X | CH 688 787 A (LINSIG DIETER ;RICHLI THOMAS (CH)) 31 March 1998 (1998-03-31) column 2, line 4,15-42,60 column 3, line 6-14,18,19,34-37; example 2 ---- | 1-39, 52-57 |
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Klaver, J

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/01589

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|---|
| X | PATENT ABSTRACTS OF JAPAN vol. 1998, no. 10, 31 August 1998 (1998-08-31) & JP 10 120519 A (OSAKA SEIYAKU:KK), 12 May 1998 (1998-05-12) abstract --- | 1-37, 52 |
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| X | WO 96 37210 A (ROPAPHARM B V I O ; NINKOV DUSAN (NL)) 28 November 1996 (1996-11-28) page 2, line 27-36 page 3, line 6-24; examples 4, 6 --- | 1-39, 52-57 |
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INTERNATIONAL SEARCH REPORT

International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| X | <p>WO 97 17944 A (AL CHI MI A S R L; CALDERINI GABRIELLA (IT); SCESA CARLA (IT)) 22 May 1997 (1997-05-22) page 1, line 15-27; example 4</p> <p style="text-align: center;">---</p> | <p>1-3, 5, 7-9, 11, 38, 39</p> |
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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 00/01589

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/01589

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| X | <p>C. REGNAULT-ROGER ET AL.: "Insecticidal effect of essential oils from mediterranean plants upon Acanthoscelides obtectus say (Coleoptera, Bruchidae), a pest of kidney bean." J. CHEM. ECOLOGY, vol. 19, no. 6, 1993, pages 1233-1244, XP000940842 see: 'Discussion' and Table 1. -----</p> | 53-57 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/01589

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
1-19, 33, 34, 38-57
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-19, 33 (partly), 34 (partly)

A composition for use in the treatment of a human or animal having a parasitic insect infestation comprising an essential oil obtained from a plant selected from the genera Artemisia, Citrus, Juniperus, Laurus, Myristica, Origanum, Piper or Aloysia in a gel carrier.

Use of a gel and essential oil as defined above in the manufacture of a composition for the treatment of a human or animal having a parasitic insect infestation.

A method of treating a human or animal suffering from a parasitic insect infestation by applying a composition as defined hereabove.

2. Claims: 20, 21, 29, 30 (partly), 33 - 37 (all partly),
52 (partly)

A composition for use in the treatment of a human or animal having a parasitic insect infestation comprising an essential oil obtained from a plant selected from the genera Artemisia, Citrus, Juniperus, Laurus, Myristica, Origanum, Piper or Aloysia in an aqueous alcoholic vehicle comprising from 0.1% - 20% v/v alcohol with water.

Use of a composition as defined above in the manufacture of a composition for the treatment of a human or animal having a parasitic insect infestation.

Method of treating a human or animal suffering from a parasitic insect infestation by applying the composition defined hereabove.

Method suitable for treating furnishing, clothing or plants to kill parasitic insects by applying the composition defined hereabove.

3. Claims: 22-28, 30 (partly), 31, 32, 33 - 37 (all partly),
52 (partly)

A composition for use in the treatment of a human or animal having a parasitic insect infestation comprising an essential oil obtained from a plant selected from the genera Artemisia, Citrus, Juniperus, Laurus, Myristica, Origanum, Piper or Aloysia in an alcohol/vegetable oil mixture comprising from 0.1% - 20% v/v alcohol.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Use of a composition as defined above in the manufacture of a composition for the treatment of a human or animal having a parasitic insect infestation.

Method of treating a human or animal suffering from a parasitic insect infestation by applying the composition defined hereabove.

Method suitable for treating furnishing, clothing or plants to kill parasitic insects by applying the composition defined hereabove.

4. Claims: 38, 39, 52 (partly)

Composition comprising an essential oil obtained from a plant selected from the genera Petergonium, Cymbopogon, Pimpinella, Myrtus (Cretian, Moroccan, organe), Lavandula, Pinus, Melaleuca, Cinnamomum, Apium, Thymus, Hyssopus, Rosmellus, Cananga, Mentle, Eucalyptus or Vitex in a gel carrier.

Method for treating plants to kill insects by applying this composition.

5. Claims: 40- 51, 52 (partly)

Composition comprising the alkaloid galanthamine for use in the treatment of a human or animal having a parasitic insect infestation.

Use of galanthamine or an extract of Narcissus in the manufacture of a composition for treating a human or animal suffering from a parasitic insect infestation.

Method of treating a human or animal suffering from a parasitic insect infestation by applying the composition defined hereabove.

Method suitable for treating furnishing, clothing or plants to kill parasitic insects by applying the composition defined hereabove.

6. Claims: 53 - 57

Composition comprising a gel carrier and one or more of the terpenes and terpenoids shown to have insecticidal activity for use in treating humans, animals, clothing, furnishing and plants (preferably selected from sabinene, p-cymene, beta-pinene, myrcene, limonene and terpinen-4-ol).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/01589

| Patent document cited in search report | | Publication date | Patent family member(s) | Publication date |
|---|---|---------------------|--|--|
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